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Transformation of humic substances in vermicomposting: Structural changes and impact on cowpea soil nutrient dynamics 16

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This study assesses the elemental and structural transformation of municipal solid waste (MSW) by vermicomposting at different stages of maturation and evaluates its nutritional potential on rhizosphere soil chemistry compared with NPK fertilizer. The structural characterization of MSW vermicompost at increasing maturity stages was determined by solid-state nuclear magnetic resonance (NMR) and Fourier Transform infrared (FT-IR) spectroscopies, while the elemental analysis was carried out using standard procedures. Thereafter, a field experiment was carried out at the Teaching and Research Farm of Obafemi Awolowo University, Nigeria, to assess the structural and elemental changes of humic acid-like substance extracted from cowpea rhizosphere soil as influenced by MSW vermicompost and NPK mineral fertilizer applied solely and at different proportions. The elemental results show a significant percentage decrease in major soil chemical parameters as the maturation period progresses. However, a notable increase was observed in N, P, and K contents in matured vermicompost. Structural analysis reveals the presence of polysaccharides, alkyl, cyclic, and aromatic compounds in the final vermicompost. Field experiments further substantiate the positive impact of MSW vermicompost on soil health. Plots receiving vermicompost, especially at 100% vermicompost (6 t/ha), had a higher relative percentage of O-Alkyl-C, Methoxyl-C, and carboxyl-C in both planting seasons compared to NPK-treated plots and control. Additionally, all vermicompost-treated plots significantly increased or improved soil pH, cation exchange capacity, soil organic carbon (SOC), and total nitrogen, which recorded the highest in the plot treated with 100% vermicompost. Hence, spectroscopic and elemental results revealed the quality, maturity, and stability of municipal solid waste vermicompost and its positive effect on soil rhizosphere, thus making the use of MSW a viable alternative to NPK fertilizer as a soil amendment.

Key words: Vermicompost, rhizosphere soil, characterization, municipal solid waste, humic substance, cowpea.

INTRODUCTION

There is an exponential increase in food demand globally, and meeting this challenge requires scaling up crop production through increased application of organo-mineral fertilizers as an immediate intervention to avert

food crises (Singh et al., 2020; Tahat et al., 2020). According to the United Nations' report in 2022, with the global population reaching 8 billion and projected to reach 9.7 billion by 2050, this calls for serious concern

(Arsenović, 2024).

This continuous population growth underscores the need for sustained application of mineral fertilizers. Ensuring adequate food supply for the growing population necessitates the ongoing use of mineral fertilizers to support agricultural productivity. However, the continuous and unguided use of mineral fertilizers toward ensuring global food security has become a threat to environmental sustainability (Adams et al., 2016; González et al., 2015). Its frequent use has been associated with leaching and downstream water contamination (Ju et al., 2006), eutrophication (Liang et al., 2006, 2007; Teodorescu et al., 2009), and greenhouse gas effects (González et al., 2015). In addition, cases of accelerated soil degradation (Adams et al., 2016) and massive nutrient mobility within the rhizosphere soil have been widely reported (Rengel and Marschner, 2005). This region is considered one of the most active interfaces on Earth, where several biochemical changes occur that significantly influence the availability, solubility, and uptake of nutrients by plants. Therefore, the availability of nutrients, especially within the rhizosphere, can pose a threat to sustainable crop production, especially in the tropics characterized by low-activity clay, thereby limiting crop productivity (Meena et al., 2017). A good understanding and management of the rhizosphere would be an advantage for optimum crop production (Buchkowski et al., 2017). Vermicompost, a nutrient-rich organic fertilizer produced through an eco-friendly process utilizing earthworms for waste management, holds great promise in improving soil health (Ding et al., 2021). It is a stable and mature product rich in macronutrients, and its application can enhance the physical, chemical, and microbiological properties of soil, promoting optimal plant growth (Srivastava et al., 2016; Bhat et al., 2017).

Despite the potential benefits of vermicompost, it is still in its premature stage in Nigeria, and there is limited research in this area, especially in the context of its potential in enriching the rhizosphere environment and nitrogen-fixing leguminous crops, hence the need for this study. The research aims to enrich the rhizosphere soil of cowpea using municipal solid waste vermicompost with a view to reducing the frequent use of mineral fertilizer, especially around the rhizosphere soil, for improved cowpea production. Therefore, the specific objectives were to:

1. Determine the structural and elemental composition of vermicompost from municipal solid waste;
2. Compare the effect of different fertilizer treatments on the elemental composition of cowpea rhizosphere soil; and
3. Determine the effect of different fertilizer treatments on Humic acid-like substances.

MATERIALS AND MEHODS

Study area

The field experiment was conducted at the Teaching and Research Farm (T&RF), Obafemi Awolowo University, Ile-Ife, Nigeria. This farm is located in the rainforest ecosystem of southwestern Nigeria, with an annual rainfall of approximately 1,500 mm, peaking in July and September. Situated at an elevation of about 224 meters above sea level, it lies between latitudes 7°32'N and 7°33'N and longitudes 4°32'E and 4°40'E. The average temperature ranges from 23.9 to 28.3°C, and the relative humidity varies from 61 to 83% during the early and late growing seasons. The soil at the experimental site originates from coarse-grained granite and gneiss, indicating significant weathering and leaching processes. According to local soil mapping, it is classified as Iwo Association. Based on the IUSS World Reference Base (WRB) 2015 system, the soil falls under the category of Ferric Acrisol.

Phase 1

Production and characterization of vermicompost from the mixture of municipal solid waste (MSW) and cow dung.

Production of vermicompost

Vermicomposting was prepared at the Teaching and Research Farm (T&RF), Obafemi Awolowo University (OAU), Ile-Ife, Department of Soil Science and Land Resources Management Unit. Cow dung and air-dried, sorted municipal solid waste (MSW) were manually mixed in a compost bin at a 4:1 (w/w) ratio (Figure 1). The substrate material was pre-decomposed for 2 weeks, with thorough turning performed after each week.

The moisture level in the substrate was maintained at 70-80% by sprinkling fresh water on alternate days. According to Sharma and Garg (2019) and Srivastava et al. (2020a, b), pre-decomposition was carried out to achieve thermal stability and eliminate volatile hazardous compounds. To bring the temperature of the compost down to 25°C after pre-decomposition, the pre-compost was opened in a clean, cool, and dry place away from direct sunlight. After that, a worm bin with dimensions of 45 cm × 25 cm × 30 cm (length × width × height) was manually filled with 1 kilogram of pre-composted organic material. Subsequently, 5 g of *Eudrilus eugeniae* (an equal-sized and non-clitellated earthworm) was introduced for further decomposition over 45 days (Goswami et al., 2018).

Characterization and total elemental analysis of organic material Instrumental characterization of vermicompost using FT-IR

The Fourier transform infrared (FT-IR) analysis of the analysis of the samples was conducted using a Nicolet™5700 spectrometer. For this analysis, 2 mg of each sample was mixed with 400 mg of potassium bromide (KBr) and then pressed under vacuum to form potassium bromide pellets. The measured bands ranged from 400 to 4000 cm⁻¹, covering a broad spectrum of infrared wavelengths and allowing for the identification and characterization of various functional groups present in the organic materials.

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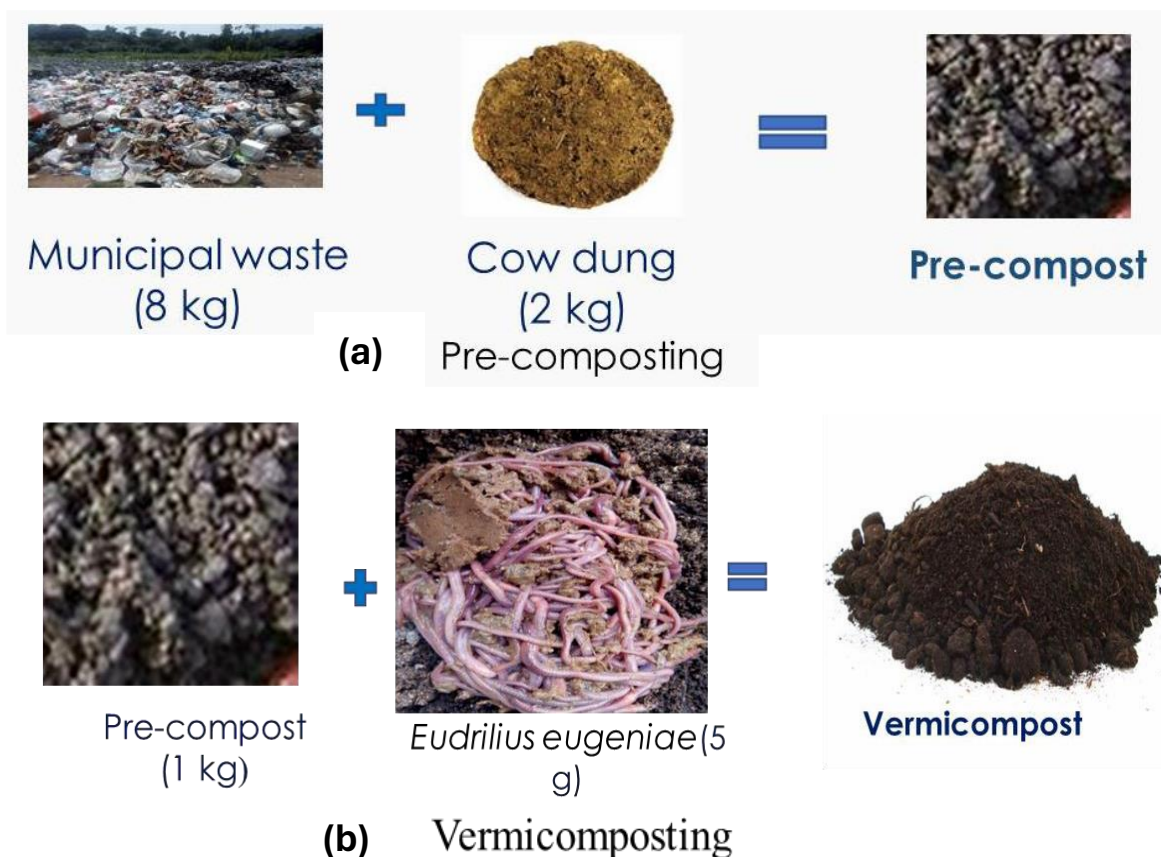


Figure 1. Pre-composting and vermicomposting of MSW: (a) pre composting of MSW and (b) vermicomposting of MSW.

NMR Characterization

This was conducted using a 300 MHz Bruker Advance wide-bore magnet equipped with a CPMAS probe. To prepare the samples for NMR analysis, freeze-dried samples weighing 60 mg were placed in 4 mm zirconia rotors and sealed with Kel-F caps. The CPMAS technique was employed, involving spinning the sample at a rate of 10 kHz at the magic-angle with respect to the magnetic field. Acquisition parameters for data collection included a recycle time of 2 seconds, a contact time of 1 millisecond, and an acquisition time of 40 milliseconds. A total of 4000 scans were collected to ensure a satisfactory signal-to-noise ratio. The ^{13}C -CPMAS-NMR spectra were interpreted by dividing the chemical shift range into different resonance regions corresponding to specific carbon environments. These regions included alkyl-C (0–45 ppm), methoxyl-C and N-C (45–60 ppm), O-alkyl-C (60–110 ppm), aromatic-C (110–160 ppm), and carboxyl- and carbonyl-C (160–190 ppm).

Phase 2

The second phase of this study was designed to achieve the second objective, which was to compare the effect of different fertilizer treatments on the elemental composition of cowpea rhizosphere soil.

Field experiment

The experimental field was conducted at the Soil Science and Land

Resources Management unit of the TandRF, OAU, Ile-Ife.

Determination of chemical properties of soil sample

Soil samples were collected randomly before planting from the 0–15 cm depth at the experimental site using a soil auger. The samples were air-dried then crushed and sieved through a 2 mm sieve for uniformity and to remove extraneous materials before sending to the laboratory. Soil pH, available phosphorus, total nitrogen, exchangeable cations and cation exchange capacity (CEC) were determined using standard procedure.

Land preparation and experimental design

Prior to planting, the land was ploughed twice and was subsequently harrowed to prepare it for sowing. The experiment was laid out in a randomized complete block design (RCBD) with nine nutrient management treatments replicated thrice. The size of each plot was 3 m × 2 m, with a 1 m alley. Two seed of Ife brown cowpea (*Vigna unguiculata*) were sown at 3 cm depth and a space of 75 × 25 cm apart resulting in approximately 133,333 plants / ha. Fertilizer treatments were applied at planting. Nutrient management treatments are as follow: T1: 120 kg N/ha as NPK 15-15-15 (Omoigui et al., 2018) farmers' practice; T2: Municipal Solid Waste Vermicompost at 100% (MSW-VC at 6 tonnes MSW/ha); T3: 75% NPK fertilizer + 25% MSW-VC; T4: 50% NPK fertilizer + 50% MSW-VC; T5: 25% NPK fertilizer + 75% MSW-VC; T6: 75% MSW-VC; T7:

50% MSW-VC; T8: 25% MSW-VC; T9: No fertilizer (control).

Other agronomic practices: control of insect pest and weeds

Cypermethrin 10% EC (300 ml/15 L) was used to control pests using a knapsack sprayer. Spraying was conducted during the 5th, 6th, and 7th weeks after planting, following the recommended rate of 12.5 L per 500 m² (Munyuli et al., 2009). Weeding was done manually twice using hand-pulling and hoeing during the second and fifth weeks after sowing. The experiment was repeated in the same field during the subsequent cropping season to assess residual effects, without additional fertilizer treatments.

Cowpea rhizosphere soil sample collection

At 50% flowering, five cowpea plants from the middle row, along with their rhizosphere soil were selected for sampling (De Feudis et al., 2016). Prior to uprooting, the plots were moistened to facilitate root lifting and minimize nodule retention in the soil. The roots were gently shaken into a bowl to collect the rhizosphere soil, which was later air-dried, sieved, and stored for chemical analysis and structural analysis.

Rhizosphere soil characterization

The soil pH and CEC were determined using the standard procedure. Organic carbon and total nitrogen content analysis involved the use of an UNICUBE elemental analyzer (Elementer Analyse system GmbH, Germany) on 20 mg soil samples; this was performed in triplicate available P by The Bray-1 method (Wuenschel et al., 2015).

Phase 3

Phase 3 was tailored towards achieving the third objective that is, determine the effect of different fertilizer treatments on Humic acid-like substances.

Extraction of humic acids from rhizosphere soil

Humic acid was extracted using the International Humic Substances Society method. Fifty grams of soil samples were mixed with a 0.1 M NaOH solution and centrifuged for 12 h. The supernatant was carefully transferred to another container, and this process was repeated until the supernatant became colorless. The extracts were acidified using concentrated HCl (6 M) to precipitate the humic acid-like substances, which were then allowed to settle for 24 h. The solution was centrifuged to separate the fulvic acid fraction. The remaining residue, representing the HALS fraction, underwent further purification through a series of dissolution and precipitation steps using 0.1 M KOH/0.2 M KCl and 0.1 M HCl/0.3 M HF solutions, repeated three times. The resulting humic acid precipitate was transferred to a Visking dialysis tube and dialyzed against deionized water until the dialysis water gave a negative Cl⁻ test with silver nitrate (AgNO₃), indicating the removal of chloride ions. Finally, the HALS fraction was freeze-dried and stored for future use.

Instrumentation and NMR method

One hundred milligrams of the freeze-dried sample was loaded into 4 mm zirconia rotors and sealed. The ¹³C-CPMAS-NMR spectra were recorded with specific parameters, including rotor spin rate, recycle time, contact time, and acquisition time. A sufficient number of scans

were collected to achieve a reliable signal-to-noise ratio. Fourier transform was performed on the data points with appropriate apodization. The spectra were interpreted by dividing the chemical shift range into distinct regions representing different carbon groups, including alkyl, aromatic, and hydrophilic carbon molecules. Structural indexes, such as the Hydrophobic index (HB) and Lignin ratio (LR), were calculated based on the relative distribution of carbon signals. The HB was determined by summing the signal intensities of apolar alkyl and aromatic carbon components and dividing by the sum of signal intensities of hydrophilic carbon molecules. The LR was calculated based on the abundance of Methoxyl-C and N-alkyl groups compared to O-aryl-C.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) to determine if there were significant differences among the treatments. In cases where the F-values were determined to be significant, Duncan's Multiple Range Test (DMRT) was used to separate the treatment means at the 5% level of probability.

RESULTS

Antecedent soil properties of the study area

The soil had the following properties as shown on Table 1; the sand, silt and clay were 736.8 g/kg, 143.2 g/kg, and 120.0 g/kg respectively, classifying it as a sandy loam texture. The pH of the soil in water was slightly acidic. The soil contained 7.7 g/kg of organic carbon, 11.62 mg/kg of available phosphorus, and 1.67 g/kg of total nitrogen. The CEC of the soil was measured at 4.25 cmol/kg.

Changes in elemental composition of vermicompost at different stages of decomposition

As shown in Table 2, the pH and total organic carbon (TOC) of the final vermicompost decreased slightly compared to the initial pre-composted waste mixture. Conversely, the total nitrogen, total phosphorus (TP), and potassium (K) content showed a significant increase in the final vermicompost (1.65%) compared to the pre-composted material. The carbon-to-nitrogen (C/N) ratio decreased significantly by 41.6% (from 30.72 to 17.94) in the final vermicompost. With the exception of zinc, the concentrations of heavy metals decreased significantly ($p \leq 0.05$) compared to the pre-composed waste mixture, with percentage decreases of 55.16% for Fe, 83.97% for Pb, 84.77% for Cr, and 75.15% for Cd.

FTIR spectra of municipal solid waste vermicompost at different stages of decomposition

As shown in Figure 2, a gradual decrease in peak intensity was observed at 3430, 2922, and 1645 cm⁻¹, corresponding to the presence of alcohol, methylene bands, and amide bands, respectively. Additionally, prominent shoulders were observed at 1034 cm⁻¹ and

Table 1. Antecedent soil properties of the experimental plot.

Physical and chemical properties	Value
Sand (g/kg)	736.8
Silt (g/kg)	143.2
Clay (g/kg)	120.0
Textural class	Sandy loam
Ph (H ₂ O)	5.74
Ph (CaCl ₂)	5.14
Soil organic carbon (g/kg)	7.70
Available phosphorus (mg/kg)	11.62
Total nitrogen (g/kg)	1.67
Exchangeable calcium (cmol/kg)	2.85
Exchangeable magnesium (cmol/kg)	0.834
Exchangeable potassium (cmol/kg)	0.07
Exchangeable sodium (cmol/kg)	0.003
Cation exchangeable capacity (cmol/kg)	3.75

Table 2. Elemental composition of municipal solid waste vermicompost at different stages of decomposition.

Parameter	15 days (Precompost)	30 days vermicompost	45 days
pH (CaCl ₂)	7.92 ^a	7.34 ^b	7.21 ^b
OC (%)	46.70 ^a	43.50 ^b	29.60 ^c
TN (%)	1.52 ^c	1.88 ^a	1.65 ^b
C/N	30.72 ^a	23.14 ^b	17.94 ^c
Total P (%)	0.79 ^c	1.12 ^b	1.52 ^a
Total K (g/kg)	1.51 ^b	1.63 ^{ab}	1.73 ^a
Fe (ppm)	45.50 ^a	25.60 ^b	20.40 ^c
Zn (ppm)	18.80 ^b	22.3 ^b	28.5 ^a
Pb (ppm)	1.56 ^a	0.78 ^{ab}	0.25 ^b
Cr (ppm)	2.56 ^a	0.56 ^b	0.39 ^c
Cd (ppm)	3.30 ^a	1.45 ^b	0.82 ^b

Means of the same alphabets along the rows are not significantly different; however, means of different alphabets are significantly different at 5% probability level according to Duncan Multiple Range Test (DMRT). OC: Organic carbon, TN: total nitrogen, C/N: carbon to nitrogen ratio.

1460 cm⁻¹. Peaks around 699 and 796 cm⁻¹ suggested the presence of alkyl halides and polysulfide groups, respectively.

The ¹³C-CPMAS-NMR spectra of vermicompost samples at different stages of decomposition (pre-compost, 30 and 45 days) are presented in Figure 3, with 84.77% for Cr, and 75.15% for Cd.

FTIR spectra of municipal solid waste vermicompost at different stages of decomposition

As shown in Figure 2, a gradual decrease in peak intensity was observed at 3430, 2922, and 1645 cm⁻¹, corresponding to the presence of alcohol, methylene bands, and amide bands, respectively. Additionally,

prominent shoulders were observed at 1034 and 1460 cm⁻¹. Peaks around 699 and 796 cm⁻¹ suggested the presence of alkyl halides and polysulfide groups, respectively.

The ¹³C-CPMAS-NMR spectra of vermicompost samples at different stages of decomposition (pre-compost, 30 and 45 days) are presented in Figure 3, with the distribution of signal areas provided in Table 3. The alkyl-C (0-45 ppm) and O-alkyl-C (60-110 ppm) regions showed strong signals, comprising approximately half of the total signal area in each sample. The second most abundant signal was within the 110-160 ppm range. A notable peak was observed around 72 ppm, representing the overlapping resonances of specific carbons in the pyranoside structure of cellulose and hemicellulose. Based on these calculations, the hydrophobicity index (HB/HI),

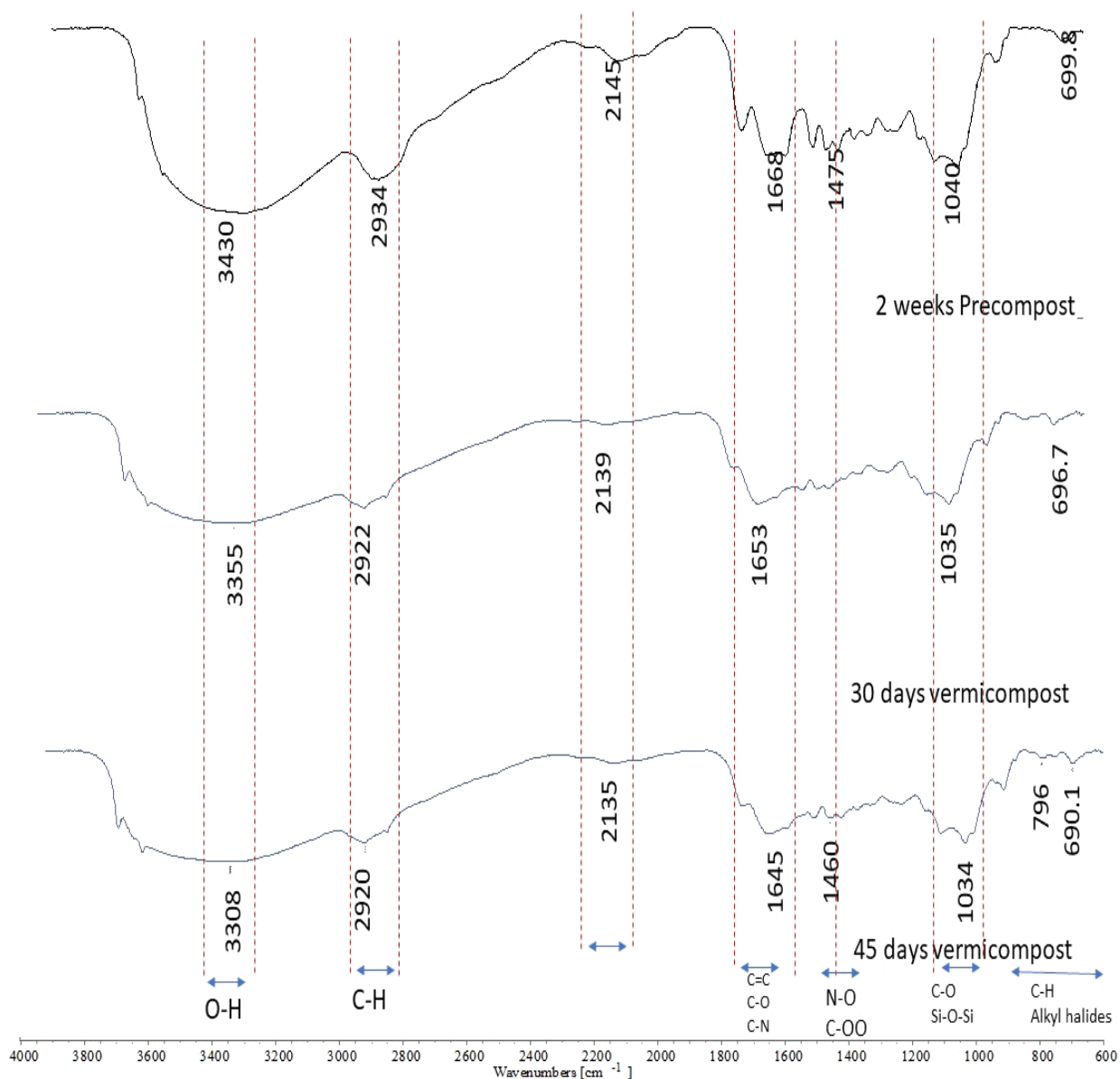


Figure 2. FTIR spectra of municipal solid waste vermicompost at different stage of decomposition.

aromaticity (ARM), and lignin ratio (LR) were determined (Table 3).

Effects of fertilizer application on chemical properties of cowpea rhizosphere soil at first planting season in 2021 and second planting season in 2022

Soil pH

All plots with vermicompost significantly ($P < 0.05$) improved soil pH with the exception of T8 which was not significantly different from T1 while T9 had the least. In the

second planting season, T2 (6.76) and T4 (6.85) had the highest pH compared to T1 (5.95) while the control (5.33) had the least (Table 4).

Total nitrogen

Plots with T2 (3.63g/kg), T4 (3.53g/kg) and T6 (3.64g/kg) had the highest total nitrogen compared to T1 (3.20 g/kg) while the control (2.00 g/kg) had the least in the first planting season (Table 4).

During the repeat experiment, T2 significantly increased the total nitrogen with 5.37 g/kg compared to T1 with 3.34

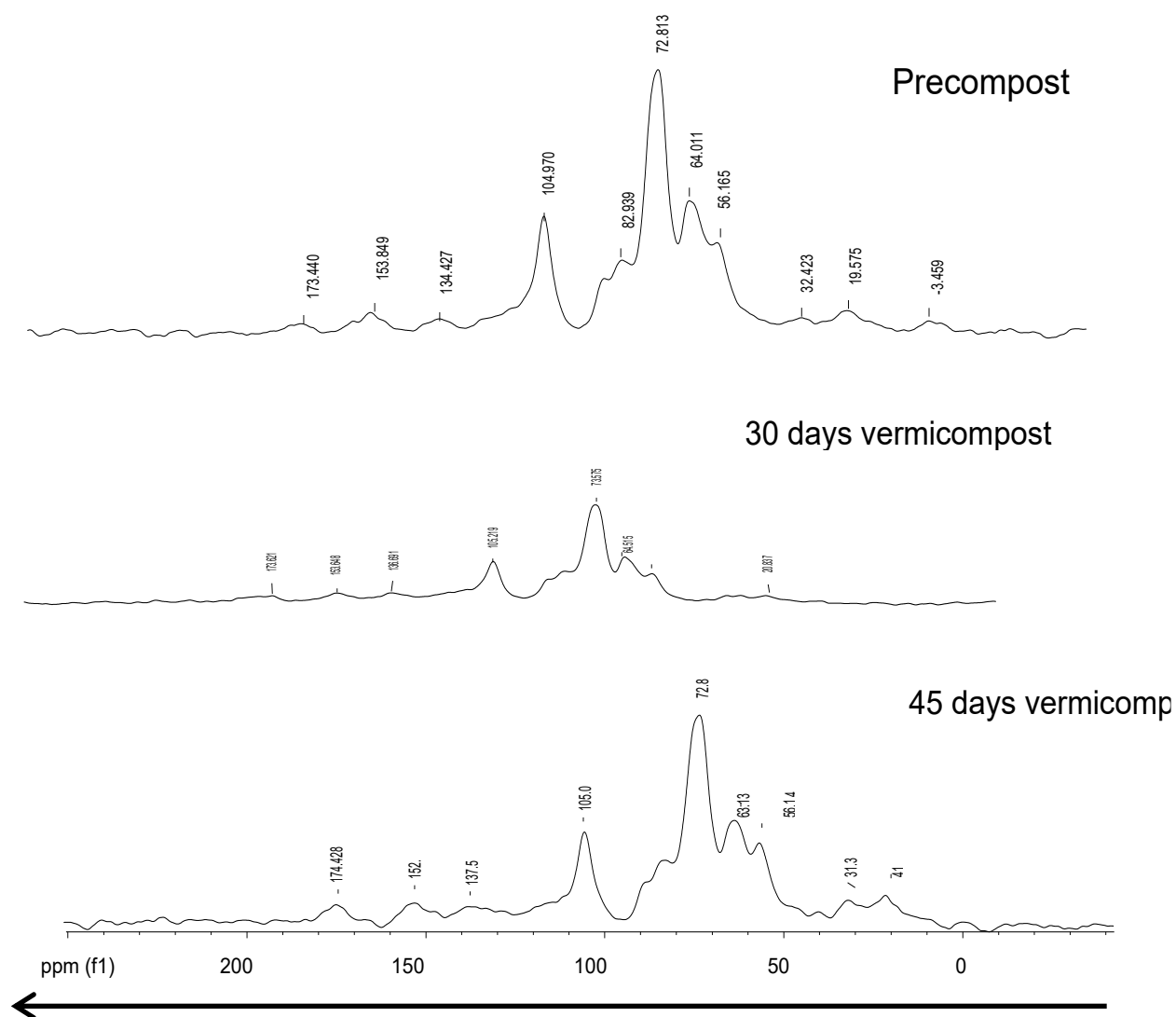


Figure 3. ^{13}C -CPMAS-NMR of vermicompost samples at different stages of decomposition (Precompost, 30 and 45 days vermicompost).

Table 3. Relative percentage distribution of signal area over chemical shift regions (ppm) in ^{13}C -CPMAS-NMR spectra of vermicompost at increasing rate of maturity

Attribute	Precompost	30 Days Vermicompost	45 Days Vermicompost
Carboxyl -C (190 -160)	3.52	6.11	7.35
Phenol -C (160 -145)	3.61	4.41	5.17
Aryl -C (145 -110)	8.27	12.13	11.95
O - Alkyl -C (110 - 60)	61.84	59.93	50.15
Methoxyl -C (60 - 45)	12.28	8.79	10.20
Alkyl -C (45 - 0)	10.47	8.63	15.09
HB	1.32	1.09	1.26
LR	3.40	1.99	1.97
A/OA	0.17	0.14	0.30

HB/HI – Hydrophobic ratio A/OA – Alkyl Ratio; LR – Lignin ratio; HB/HI = $\Sigma [(0-45) + (45-60)/2 + (110-160)] / \Sigma [(45-60)/2 + (60-110) + (160-190)]$ A/OA = $(0-45) / (60-110)$; LR = $(45-60) / (145-160)$.

Table 4. Effects of fertilizer application on chemical properties of the rhizosphere soil in 2021 and 2022 planting seasons.

Treatment	pH (CaCl ₂)	OC (g/kg)	TN (g/kg)	Av. P(mg/kg)	Ex. K cmol/kg	CEC (cmol/kg)	pH (CaCl ₂)	OC (g/kg)	TN (g/kg)	Av. P(mg/kg)	Ex. K cmol/kg	CEC (cmol/kg)
2021						2022						
T1	-	7.89 ^g	3.20 ^b	11.25 ^{cd}	0.08 ^{cde}	4.89 ^d	5.95 ^c	7.60 ^f	3.34 ^{cd}	11.51 ^e	0.08 ^{cde}	5.93 ^b
T2	-	12.9 ^{bc}	3.63 ^a	15.03 ^a	0.11 ^a	6.00 ^{abc}	6.76 ^a	15.52 ^a	5.37 ^a	16.13 ^a	0.16 ^a	7.67 ^a
T3	-	8.90 ^f	3.13 ^b	11.72 ^{cd}	0.08 ^{de}	5.24 ^{bcd}	5.85 ^c	13.34 ^{cd}	3.34 ^{cd}	11.55 ^e	0.08 ^{de}	6.11 ^b
T4	-	13.70 ^a	3.57 ^a	13.70 ^b	0.09 ^{cd}	5.49 ^{abcd}	6.85 ^a	14.73 ^b	4.73 ^b	14.06 ^c	0.11 ^{bc}	6.60 ^{ab}
T5	-	11.78 ^d	3.47 ^{ab}	13.97 ^b	0.09 ^{bc}	5.84 ^{abcd}	6.42 ^b	14.78 ^{ab}	4.78 ^{ab}	14.66 ^{bc}	0.13 ^b	6.60 ^{ab}
T6	-	13.30 ^{ab}	3.64 ^a	13.52 ^b	0.10 ^{ab}	6.26 ^{ab}	6.44 ^b	15.11 ^{ab}	5.11 ^{ab}	15.00 ^b	0.13 ^b	7.20 ^{ab}
T7	-	10.93 ^e	3.30 ^{ab}	11.94 ^c	0.08 ^{cde}	6.39 ^a	6.00 ^c	14.89 ^{ab}	4.48 ^{ab}	12.85 ^d	0.13 ^b	6.97 ^{ab}
T8	-	12.50 ^c	2.50 ^C	10.90 ^d	0.07 ^{ef}	5.36 ^{abcd}	5.95 ^c	13.63 ^c	3.63 ^c	11.67 ^e	0.09 ^{cd}	6.10 ^b
T9	-	7.37 ^g	2.00 ^d	9.77 ^e	0.07 ^f	5.08 ^{cd}	5.33 ^d	7.90 ^f	2.90 ^d	11.03 ^e	0.07 ^e	6.03 ^b
Initial soil properties	-	7.70	31.67	11.62	0.07	3.75	5.74	7.70	1.67	11.62	0.07	3.75

Means of the same alphabets down the column are not significantly different; however, means of different alphabets are significantly different at 5% probability level according to Duncan Multiple Range Test (DMRT). WAP = weeks after planting. T1 =100% NPK T2=100% VC; T3= 25%NPK+75%VC T4= 50%NPK+50%VC T5= 25%NPK+75%VC T6= 75% VC; T7= 5Control.

g/kg which was not significantly different from the control (2.90 g/kg).

Soil organic carbon

Plot with T4 significantly influenced a higher soil organic carbon (SOC) during the first planting season with 13.70 g/kg compared to T1 with 7.89 g/kg which was not significantly different from T9 (7.37 g/kg). A significant higher value was recorded in T2 (15.52 g/kg), though not significantly different from T5 (14.78 g/kg), T6 (15.11 g/kg), and T4 (14.73 g/kg) treated plots in the second planting season but performed better than T1 (7.6 g/kg) and T9 (7.9 g/kg) significantly (Table 4).

Available phosphorus and CEC

Plot with T2 has the highest available P compared

T1 in the first planting season, similar trend was observed in the second planting season (Table 4). CEC increased significantly in all plots treated with vermicompost compared to T1 which was not statistically different from T9 in the first planting season.

¹³C-CPMAS-NMR of humic acid – like substance

The relative distribution of signal areas over chemical shift is presented in Tables 5 and 6, enabling the calculation of parameters such as HB/HI, ARM, and LR. The result revealed the prevalence of Alkyl-C (0–45 ppm) and O-Alkyl-C (60–110 ppm) regions, which accounted for approximately 50% of the total signal area in each sample (Table 3). Aromatic carbons within the 110–160 ppm range were identified as the second most abundant compounds in all humic extracts (Table 5). Similarly, the methoxyl-C (45–60 ppm) and

carbonyl/carboxyl-C (160–190 ppm) intervals displayed notable signals Figure 4 and 5.

DISCUSSION

Changes in elemental composition of vermicompost at different stages of decomposition

The notable decline in pH during the vermicomposting can primarily arise from the neutralization of carboxylic and phenolic groups of humic acids by calcium and ammonia, which are secreted by earthworms during vermicomposting (Sharma and Garg, 2017, 2018). This decrease is advantageous as it aids in nitrogen preservation within the vermicompost (Sharma and Garg, 2017; Arora and Kaur, 2019). Another notable is the decline in TOC; this can be due to the degradation and mineralization of organic matter. The presence

Table 5. Relative percentage distribution of signal area over chemical shift regions (ppm) in¹³C- CPMAS-NMR spectra of humic acids extracted from cowpea rhizosphere soil 2021 planting season.

Attribute	Carboxyl-C (190 – 160)	Phenol – C (160 – 145)	Aryl – C (145 – 110)	O – Alkyl – C (110 – 60)	Methoxyl – C (60 – 45)	Akyl – C (45 – 0)	HB/HI	A/OA	LR
T1	12.81	4.97	18.23	17.16	10.20	24.78	1.09	0.44	0.62
T2	15.14	6.40	20.75	27.95	16.50	33.05	1.35	1.18	2.57
T3	12.71	5.10	18.70	18.13	10.60	20.70	1.19	1.14	1.07
T4	15.10	5.50	19.20	18.20	12.10	29.90	1.32	1.64	2.20
T5	15.10	5.50	19.20	18.20	12.10	30.00	1.32	1.65	2.20
T6	13.90	6.20	20.10	28.30	16.40	28.20	1.30	0.99	2.64
T7	13.50	6.30	19.40	24.00	12.30	24.40	1.24	1.01	1.95
T8	12.40	5.10	21.20	24.70	12.10	24.50	1.27	0.99	2.37
T9	12.40	4.30	17.57	24.70	9.50	24.50	1.31	0.99	0.60

HB/HI = Hydrophobic ratio; A/OA = Alkyl ratio; LR = Lignin ratio; HB/HI = $\Sigma [(0 - 45) + (45 - 60) / 2 + (110 - 160)] / \Sigma [(45 - 60) / 2 + (60 - 110) + (160 - 190)]$; A/OA = $(0 - 45) / (60 - 110)$; LR = $(45 - 60) / (145 - 160)$. T1 = 100% NPK; T2 = 100% MSW-VC; T3 = 75%NPK + 25% MSW-VC; T4 = 50% NPK + 50% MSW-VC; T5 = 25% NPK + 75% MSW-VC; T6 = 75% MSW-VC; T7 = 50% MSW-VC, T8 = 25% MSW-VC, T9 = control.

Table 6. Relative percentage distribution of signal area over chemical shift regions (ppm) in¹³C- CPMAS-NMR spectra of humic acids extracted from cowpea rhizosphere soil in 2022 season.

Attribute	Carboxyl-C (190 – 160)	Phenol – C (160 – 145)	Aryl – C (145 – 110)	O – Alkyl – C (110–160)	Methoxyl – C (60 – 45)	Akyl – C (45 – 0)	HB/HI	A/OA	LR
T1	13.70	3.15	14.34	15.41	3.08	30.10	1.00	0.95	0.97
T2	29.90	8.96	25.06	25.40	15.30	25.40	1.50	1.59	2.70
T3	11.99	5.60	20.99	15.34	11.97	23.57	1.25	1.35	2.13
T4	13.25	4.73	19.42	17.38	12.23	28.38	1.34	1.63	2.58
T5	17.44	5.01	24.03	23.14	11.95	15.05	0.95	0.65	2.38
T6	17.30	4.10	19.90	28.80	11.60	27.20	1.21	0.94	2.82
T7	25.13	8.90	12.10	15.80	7.60	25.80	0.92	1.63	0.85
T8	13.60	4.70	16.80	29.80	10.90	25.90	1.30	0.86	2.31
T9	11.80	4.10	16.20	20.40	8.40	28.00	0.44	0.37	0.34

HB/HI = Hydrophobic ratio; A/OA = Alkyl ratio; LR = Lignin ratio; HB/HI = $\Sigma [(0 - 45) + (45 - 60) / 2 + (110 - 160)] / \Sigma [(45 - 60) / 2 + (60 - 110) + (160 - 190)]$; A/OA = $(0 - 45) / (60 - 110)$; LR = $(45 - 60) / (145 - 160)$. T1 = 100% NPK; T2 = 100% MSW-VC; T3 = 75%NPK + 25% MSW-VC; T4 = 50% NPK + 50% MSW-VC; T5 = 25% NPK + 75% MSW-VC; T6 = 75% MSW-VC; T7 = 50% MSW-VC, T8 = 25% MSW-VC, T9 = control.

of microbial populations and earthworms in the vermicompost system actively contributes to respiratory activity and carbon assimilation, resulting in a decrease in the organic carbon content, indicating the enrichment of humic acids (Goswami et al., 2016; Karmegam et al., 2019). The observed increase in total nitrogen (TN) content in the final vermicompost can be attributed to several factors. Firstly, the mineralization of nitrogenous organic substrates, particularly proteins, during the vermicomposting process contributes to the release of nitrogen in the form of ammonium (NH₄⁺) and nitrates (NO₃⁻) (Gómez-Brandón et al., 2013). Additionally, the activity of nitrogen-fixing bacteria and other microorganisms within the vermicompost system can contribute to the increase in total nitrogen content (Sharma and Garg, 2019). Furthermore, the excretory substances of earthworms, such as body fluids, coelomic fluids

(mucus), growth- stimulating hormones, enzymes, and the decomposition of dead earthworms, can also contribute to the overall nitrogen content of the vermicompost (Arumugam et al., 2018).

There was also an observed increase in total TP and K content in the final vermicompost, this can be attributed to specific processes during vermicomposting. Phosphorus mineralization is facilitated by the enzymes present in the gut of the earthworm which aid the breakdown of organic phosphorus compounds into inorganic forms that are readily available for plant uptake (Hanc and Pliva, 2013; Suthar et al., 2015). Similarly, potassium mineralization in vermicompost is primarily driven by endo- or exogenic enzymes present in earthworm intestines. These enzymes facilitate the conversion of organic potassium compounds into inorganic forms, thereby increasing the availability of potassium in the vermicompost (Yadav et al., 2013).

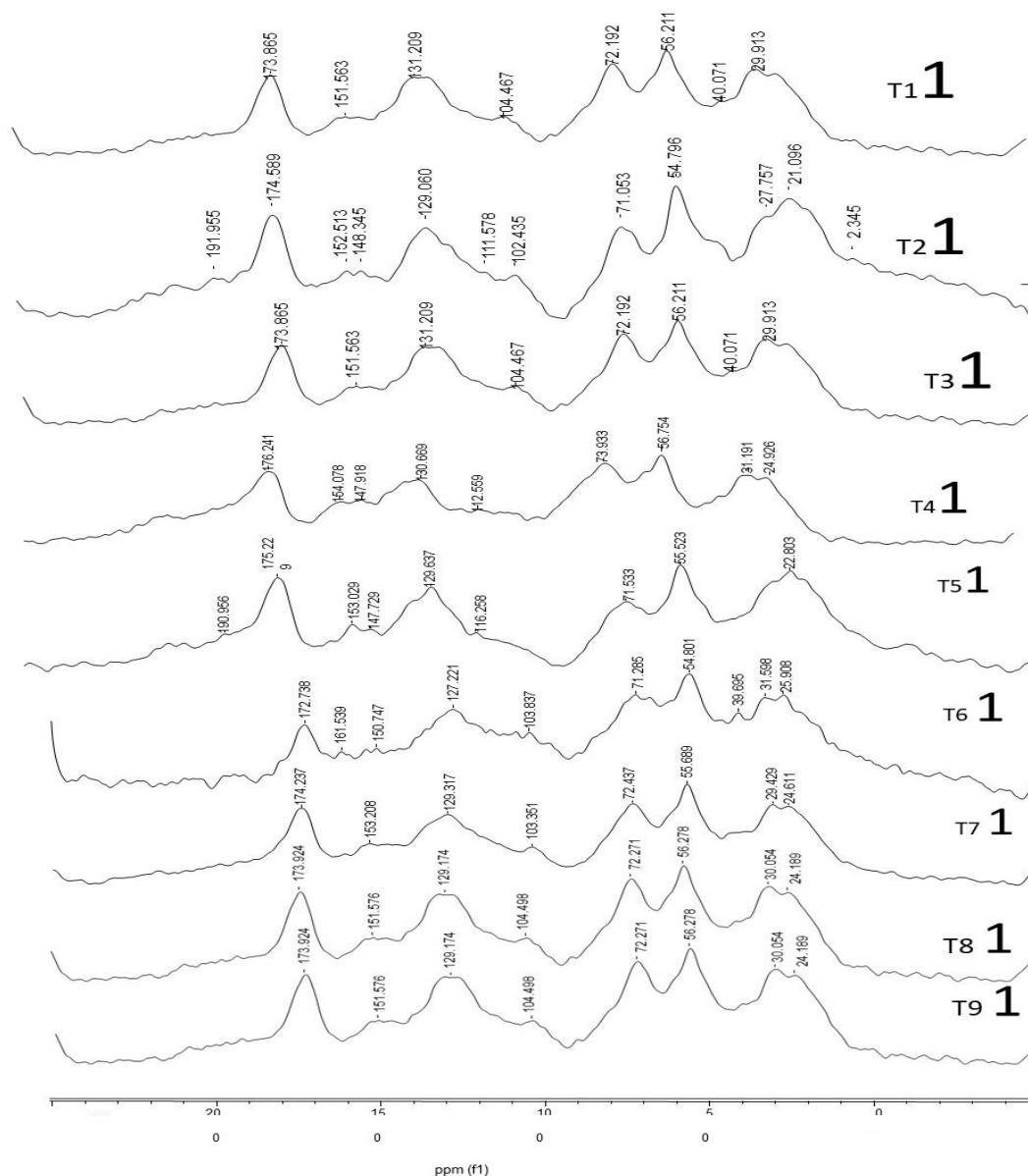


Figure 4. ^{13}C -CPMAS-NMR Spectra for humic acid of cowpea rhizosphere soil under different fertilizer treatment (2021). 1= First planting season, T1=100% NPK; T2= 100% MSW-VC; T3= 75%NPK+25% MSW-VC; T4= 50% NPK + 50% MSW-VC; T5= 25% NPK+ 75% MSW-VC; T6 = 75% MSW-VC; T7= 50% MSW-VC, T8= 25% MSW-VC, T9= Control.

C/N ratio

The observed decrease during vermicomposting can be attributed to the breakdown and mineralization of organic matter. Through microbial activity and decomposition, carbon is lost while nitrogen becomes enriched, resulting in a lower C/N ratio (Malafaia et al., 2015). Additionally, the humification process in vermicomposting leads to the decomposition of complex substances like lignin, cellulose, and hemicellulose, which are high in carbon content (Das and Bhattacharya, 2016). C/N ratio below 20

is generally considered an indicator of mature vermicompost (Morais and Queda, 2003).

Heavy metal content

The decrease in heavy metals such as Cd, Cr, Pb, and Fe content, in the final vermicompost compared to the initial feed mixture (precompost) can be attributed to the detoxification mechanisms exhibited by earthworms. Earthworms have the remarkable capability to accumulate

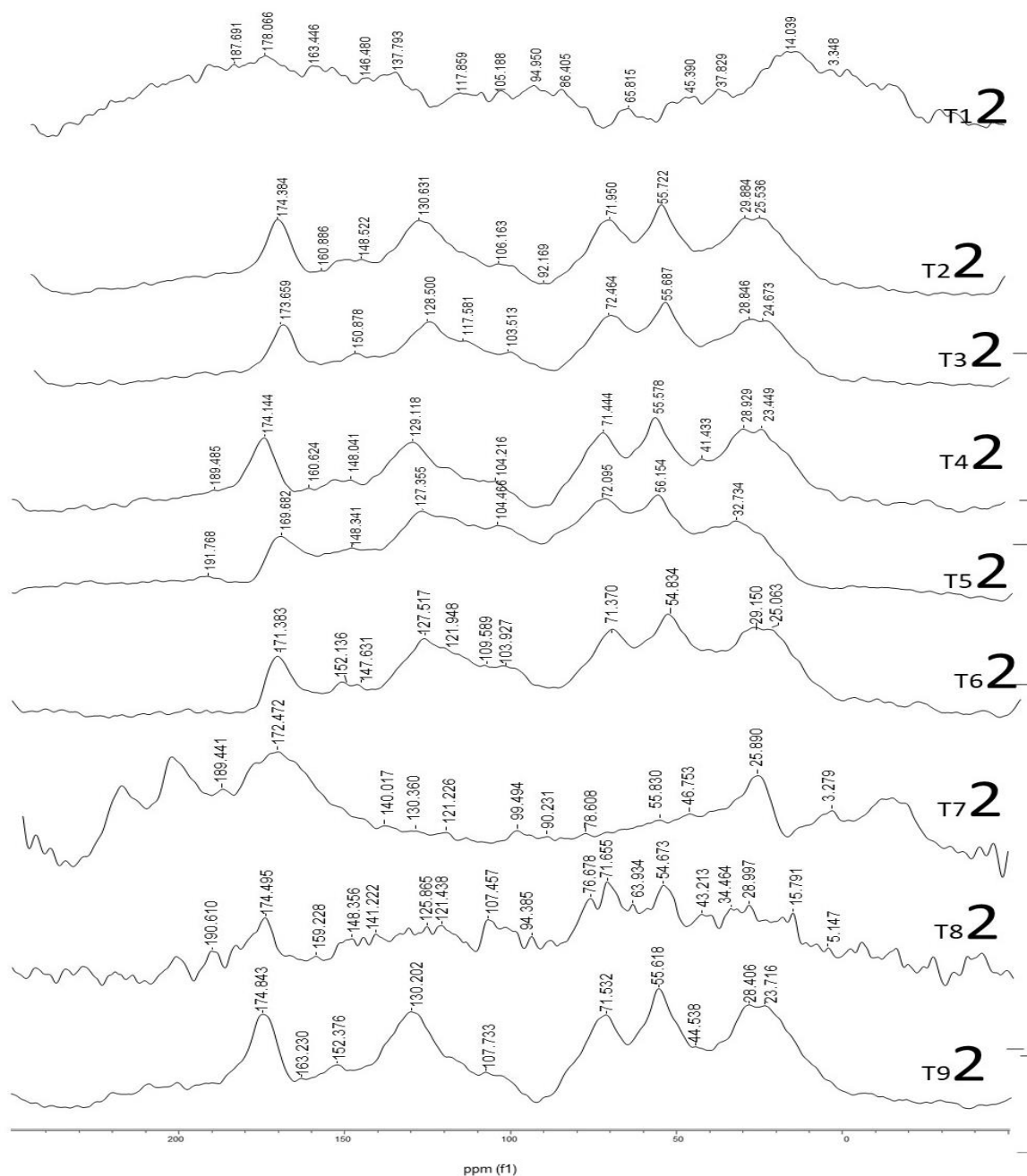


Figure 5. ^{13}C -CPMAS-NMR Spectra for humic acid of cowpea rhizosphere soil under different fertilizer treatment (2021). 2= Second planting season, T1=100% NPK; T2= 100% MSW-VC; T3= 75%NPK+25% MSW-VC; T4= 50% NPK + 50% MSW-VC; T5= 25% NPK+ 75% MSW-VC; T6= 75% MSW-VC; T7= 50% MSW-VC, T8= 25% MSW-VC, T9= Control.

and immobilize heavy metals in specific tissues, such as chloragocytes and intestinal microbiota (Maity et al., 2009). Metallothionein (MTs), a heavy metal-binding protein found within the internal body of earthworms, plays a pivotal role in the detoxification process (Azizi et al., 2015). Research has indicated that earthworms can effectively reduce the concentration of heavy metals

during the vermicomposting process (Azizi et al., 2015).

Fourier transforms infrared analysis of vermicompost at different stages of decomposition

A decrease in peak intensity at 3430 cm^{-1} indicate the

breakdown of phenols and carbohydrates, this finding is in line with previous research by Lim et al (2016) on palm oil mill effluent vermicompost, where a decline in peak intensity at 3328 cm indicated carbohydrate degradation. Similarly, Hussain et al. (2015) observed a significant reduction in peak intensity at 3448 cm, suggesting a decrease in phenolic content. The decrease in phenolic content during vermicomposting indicates favourable vermicompost quality, characterized by the breakdown of inhibitory compounds, enhanced nutrient availability, improved microbial activity, and reduced phytotoxicity (Gajalakshmi and Abbasi, 2004; Hussain et al., 2015). Peaks around 2924 and 2920 cm^{-1} indicated the degradation of lipids and carbohydrates, corresponding to a decrease in aliphatic structures. This observation aligns with the findings of Droussi et al. (2009), who reported a decrease in methylene bands (C-H stretch) at 2920 and 2850 cm. The breakdown of complex organic compounds in the vermicompost leads to the enrichment of simpler, more readily available organic matter (Zaccheo et al., 2002). Additionally, the degradation of aliphatic structures and the reduction of polysaccharides and carbohydrates contribute to a decrease in the C/N ratio of the vermicompost.

The presence of peaks at 1668 and 1645 cm^{-1} indicates the presence of peptidic material with amide I and amide II bonds, which contribute to the total nitrogen content of the vermicompost. This observation is consistent with the reported increase in total nitrogen in the elemental composition analysis of the vermicompost. Carbohydrate presence was confirmed by peaks at 1034, 1035, and 1040 cm^{-1} , corresponding to C-O bonds in polyalcoholic and ether functional groups typically found in oligo- and polysaccharides. The content of lipid compounds remained relatively stable throughout the vermicomposting process, as indicated by peaks at 2934-2920 and 1475 cm^{-1} . Peaks around 699 and 796 cm^{-1} can be attributed to alkyl halides and polysulfide groups (Fuchs et al., 2011; Reusch and Boyd, 2013).

^{13}C -CPMAS-NMR of vermicompost samples

The alkyl-C (0–45 ppm) and O-alkyl-C (60–110 ppm) regions of the initial waste mixture (Precompost) indicated a predominance of alkyl and sugar components (Figure 3). The O-alkyl-C region (60–110 ppm) showed resonances attributed to monomeric units in oligo- and polysaccharide chains, indicating the potential contribution of the vermicompost to soil organic matter (SOM) (Johnson et al., 2005). Notably, a strong signal at around 72 ppm corresponded to the pyranoside structure of cellulose and hemicellulose, which are easily biodegradable by microorganisms and contribute to the availability of plant nutrients (Schoning et al., 2005; Solomon et al., 2010). Distinct peaks and shoulders at various chemical shifts in the NMR spectrum indicated specific functional groups

and molecular structures. Signals at 56 ppm suggested the presence of methoxy substituents on aromatic rings of lignin structures or C–N bonds in amino acid moieties. Similarly, signals in the phenolic aromatic region (145–160 ppm) indicated the presence of O-substituted ring carbons coupled with methoxy substituents in lignin components. These observations are consistent with previous studies (Quideau et al., 2001; Dignac et al., 2002). Furthermore, the increasing broad band observed around 135–137 ppm in the vermicompost samples may be associated with alkyl substitutions in the p-hydroxy phenyl ring of cinnamic and p-coumaric units of lignin and suberin biopolymers. The high phenolic content in the 45 days vermicompost suggests the recalcitrant nature of the organic material and its potential to contribute to SOM (Yu et al., 2015; Pane et al., 2013).

Moreover, a prominent signal at 174 ppm in the 45 days vermicompost indicated a significant content of carboxyl groups, which are aliphatic acids of plant and microbial origins. Carboxyl C is considered an important pathway for dissolved organic matter production, organic matter accumulation, and soil fertility potential (Kramer et al., 2012; Anda et al., 2013).

Effect of fertilizer application on chemical properties of cowpea rhizosphere soil

The observed increase in the soil pH in both planting seasons indicated that, addition of Vermicompost (T2) and also at reduced rate with mineral fertilizer was effective in increasing soil pH. It specifically affects plant nutrient availability by controlling the chemical forms of the nutrients. The status of soil available P was also greatly enhanced as compared to initial P before sowing (Table 4), indicated that Vermicompost enhanced phosphorous concentration and uptake in soil, increasing the solubilisation of phosphorous either by microorganism activation with excretion of organic acids likes citric, glutamic, tartaric, succinic, lactic, oxalic, malic and fumaric (Sainz et al., 1998).

The increase in the CEC observed with plots treated with vermicompost is in accordance with the findings of (Marinari et al., 2000). CEC increases with increasing soil OM (Saikh et al., 1998). It is strongly affected by the nature and amount of mineral and organic colloids present in the soil. Thus, CEC measurements are commonly made as part of the overall assessment of the potential fertility of a soil, and possible response to fertilizer application (Landon, 1991).

^{13}C -CPMAS-NMR of humic acids extracted from cowpea rhizosphere soil in the 2021 and 2022

The predominance of alkyl-C and O-alkyl-C in the spectra suggests that the humic extracts were rich in aliphatic

compounds. This indicates the presence of organic matter derived from plant residues and microbial activity, which contribute to soil fertility and nutrient cycling (Spaccini and Piccolo, 2007). The presence of aromatic/phenolic carbons in all the humic extracts indicates the presence of partially degraded lignin structures and condensed aromatic compounds. These compounds contribute to the formation of stable SOM and play a role in carbon sequestration, enhancing soil fertility and structure (Spaccini and Piccolo, 2009).

The presence of carbohydrates and compounds derived from cellulose and hemicelluloses, as indicated by the signals at 71-73 ppm, suggests the presence of plant-derived organic matter that can contribute to soil structure and nutrient availability (Spaccini and Piccolo, 2009).

The variations observed in the spectra among different fertilizer treatments provide insights into the impact of these treatments on the molecular composition of humic substances. For example, the higher abundance of carbons in methoxyl groups or linked to nitrogen in T2 suggests a specific influence of this treatment on the composition of humic substances. Furthermore, the slight increase in HB/Hi and ARM index values in the humic extracts of T2 indicates an enhancement in the stability of SOM. This suggests that the addition of vermicompost as an organic fertilizer can promote the accumulation of stable organic compounds in the soil, leading to improved soil quality and nutrient cycling (Piccolo et al., 2018b; Zohaib et al., 2014; Amb and Ahluwalia, 2016; Jandl et al., 2012).

Soil organic carbon fractions of humic acid extracted from cowpea rhizosphere soil as influenced by different fertilizer treatments

O-alkyl C

The presence of O-alkyl C in the humic extracts indicates the potential contribution of these compounds to the SOM pool. In the case of T2, which showed a high amount of O-alkyl C, it suggests that vermicompost, being rich in organic inputs, has the potential to significantly contribute to SOM (Wang et al., 2013; Yu et al., 2015; Li et al., 2017; He et al., 2018; Guan et al., 2018). The decrease in the O-alkyl C fraction observed in T1 compared to the control (T9) indicates a larger proportion of cellulose and hemicellulose in the humic substances (Schoning et al., 2005; Solomon et al., 2010). The integration of vermicompost with inorganic inputs showed no relative difference in the O-alkyl C content compared to the T9.

Alkyl C

Alkyl C, which is a recalcitrant form of carbon, is known for its stability and hydrophobic nature (Carrington et al.,

2012; Chen et al., 2013; Habte et al., 2013; Yu et al., 2015). In the context of this study, the vermicompost supplied a lesser proportion of alkyl C compared to O-alkyl C, indicating that during the vermicomposting process; the stable alkyl C remains intact while the carbohydrate C (O-alkyl C) undergoes decomposition. As a result, the degradation of labile O-alkyl C leads to the accumulation of alkyl C and aromatic C (Quideau et al., 2001).

Methoxyl C

The high contents of methoxyl C observed in T2 and T6 during the first planting season suggest that the vermicompost used in these treatments had a high concentration of lignin and polyphenols in its dietary composition. The presence of high levels of methoxyl C indicates the presence of recalcitrant organic compounds derived from lignin and phenolic compounds in the vermicompost. These compounds are known to be relatively resistant to microbial degradation, thus potentially contributing to the stability and persistence of SOM in the soil.

Carboxyl C

Carboxyl C, which is an aliphatic acid derived from plant and microbial sources, was found to be relatively abundant in T2 of the humic acid extracted from cowpea rhizosphere soil in both planting seasons (Yu et al., 2015). Carboxyl C is a constituent of organic inputs and can also be generated by microbial activity. Carboxyl-rich compounds are oxidation products of plant-derived biomolecules, such as lignin and associated phenolic substances (Kramer et al., 2012). The high amounts of carboxyl C observed in the humic acid from T2 treatment in both planting seasons indicate the significant potential of vermicompost treatment to enrich the SOM. In contrast, the humic acid extracted from the rhizosphere soil of T1 showed a decreased carboxyl C content, which could potentially have a detrimental effect on soil carbon stocks. This decrease may be attributed to factors specific to the treatment or other interactions within the soil system.

Phenolic C

Phenolic C, characterized by the presence of diester P and amide N, represents a less humified organic material in SOM (Wissing et al., 2013). It is derived from recalcitrant plant litter compounds and has a slower degradation rate compared to other carbon fractions (Rumpel et al., 2004; Min et al., 2015). Therefore, the higher phenolic contents observed in the humic acid extracted from the rhizosphere soil of T2 compared to T1 and T9 treatments indicate its significant potential contribution to SOM. The high

phenolic C content also reflects the limited microbial degradation due to the recalcitrant characteristics of the organic inputs (Pane et al., 2013).

Conclusion

The results conclude that the vermicompost was rich in nutrient elements, enhancing SOM and reducing inherent heavy metal contents in municipal solid waste, thereby producing a clean organic fertilizer. The application of municipal solid waste vermicompost improved the chemical properties of the rhizosphere soil over two planting seasons.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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